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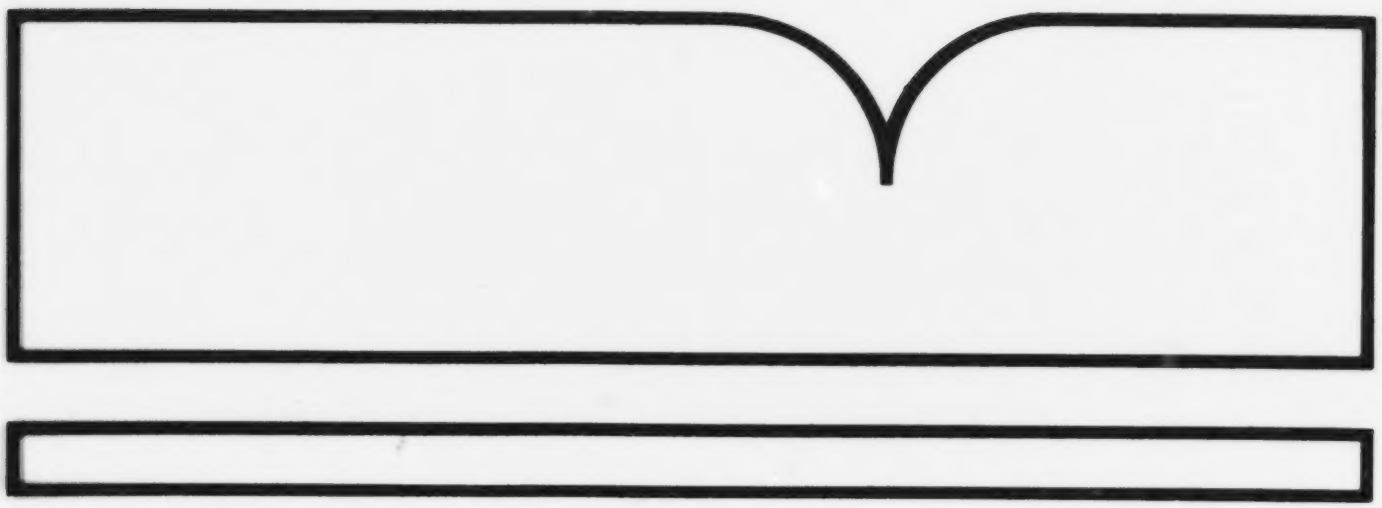

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Enhancement of Experimental Respiratory Infection following Nickel Inhalation[1]

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The effects of nickel chloride inhalation on the enhancement of experimentally induced streptococcal infections were studied. Exposure to nickel chloride (499.2 μg Ni/m³) for 2 hr produced significant enhancement in mortality. This response was observed for 24 hr after the nickel chloride exposure. A delayed clearance response of inhaled streptococci, also observed in the nickel-treated mice, supported the mortality data. Several parameters involving pulmonary cells obtained by *in situ* lavage 24 hr after nickel chloride exposure were also studied; no reduction in total cell yield, viability, or cellular composition was observed. A significant reduction in phagocytic capability, as compared to that of control animal cells, was observed in alveolar macrophages obtained 24 hr after nickel chloride exposure.

INTRODUCTION

The polluted air of many industrial metropolitan areas contains various potentially hazardous respirable trace metals including nickel (Schroeder, 1970). High-temperature combustion processes of petroleum products and coal represent the major sources of nickel (Ni) in ambient air (Bowen, 1966; Hickey *et al.*, 1967), which predominates as submicrometer-sized particulates (Lee and von Lehmden, 1973; Natusch *et al.*, 1974).

The impact of these particles on the functional integrity of the alveolar regions of the lung is extremely important. Wehner *et al.* (1975) reviewed the toxicity of nickel and its compounds and reported that the majority of nickel-related lesions were caused by inhalation exposure. Nickel has been shown to alter the functional integrity of and to be cytotoxic to alveolar macrophages (Graham *et al.*, 1975a; Waters *et al.*, 1975a). The relationship between this toxicity and respiratory disease has not been reported to date. Thus, the objectives of this study were twofold: (1) to evaluate the *in vivo* inhalation toxicity of nickel, and (2) to investigate this toxicity in terms of altered host defense mechanisms against infectious agents. In the experiments, nickel inhalation was coordinated with a laboratory-induced respiratory infection and various parameters measuring dysfunction in some respiratory defense systems were investigated. Similar studies were previously reported for cadmium (Gardner *et al.*, 1977), nickel oxide (Port *et al.*, 1975), and manganese dioxide (Maigetter *et al.*, 1976).

[1] This report has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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MATERIALS AND METHODS

Animals

Female, 4- to 6-week-old, outbred albino, strain CD-1 mice (Charles River, Wilmington, Mass.) were used throughout this investigation. The animals were housed in plastic cages on wood shavings and provided antibiotic-free Wayne Lab-Blox chow and water *ad libidum*.

In addition, female, Sprague–Dawley, albino rats (Charles River) weighing from 250 to 350 g were used. The animals were housed as mentioned previously for mice and provided antibiotic-free Purina Laboratory Chow and water *ad libidum*.

Aerogenic Administration of Nickel

In vivo inhalation exposures of rats and mice were performed as previously described (Gardner *et al.*, 1977) and involved single 2 hr exposures to a nickel chloride aerosol ($NiCl_2 \cdot 6H_2O$, certified grade, Fisher Scientific Co., Fair Lawn, N.J.) or to a nickel sulfate aerosol ($NiSO_4 \cdot 6H_2O$, certified grade, Fisher Scientific Co.). The physical description and operational details are the same as previously reported (Gardner *et al.*, 1977), with the exception that either $NiCl_2 \cdot 6H_2O$ or $NiSO_4 \cdot 6H_2O$ was used as the source of the nickel aerosol. Particle size analysis was performed during each exposure using a Royco aerosol particle monitor (Model 225, Royco Instruments, Inc., Menlo Park, Calif.) equipped with a Model 507 module. The particle size analysis indicated that 86 to 96% of the particles were $<1.4\ \mu$m diameter and at least 99% were $<3.0\ \mu$m. The temperature and humidity of the exposure atmospheres in the chamber were monitored continuously with a Rustrak temperature and relative humidity sensor (Model 225, Gulton Industries, Inc., Manchester, N.H.). The average chamber temperature was 23.5°C with a standard error of 0.1°C, while the relative humidity was 40.3 ± 0.6%.

Nickel Quantitation

During the exposure period, filter samples (0.22 μm, Millipore Corp., Bedford, Mass.) for aerosol mass quantitation of nickel were collected at regular intervals for 7 min at 2.65 liter/min. Filters were treated as previously described (Gardner *et al.*, 1977) for eluting sampled nickel.

Lungs were excised at the carina, immediately and at various time intervals postexposure, from randomly selected animals sacrificed by cervical dislocation (mice) or intraperitoneal injection (rats) of 150 mg Nembutal (sodium pentabarbital, Abbott Laboratories, North Chicago, Ill.). The lungs were placed in individually tared Pyrex boats and immediately weighed to determine wet weight. Samples were minced, oven-dried under vacuum (>90°C, 24 in. Hg) for 24 to 72 hr and low-temperature ashed (Model LTA-505, LFE Corp., low-temperature asher) at 400 RF W and 200 ml/min oxygen flow. The dried residue was dissolved with either 2 or 5 ml 6 *N* nitric acid (reagent grade, Fisher Scientific Co.) and stored at −20°C in polyethylene vials until analyzed.

Nickel quantitation was performed at 232 nm using a Perkin–Elmer atomic absorption spectrophotometer (Model 306) equipped with a heated graphite accessory (Model 2100). The following conditions were used during analysis: slit size, 7

A; drying for 50 sec, 150°C; ashing for 50 sec, 1200°C; atomizing for 9 sec, 2700°C; nitrogen flow, 50 psi; and background correction. Sample nickel quantitation was determined by interpolation on a multipoint standard curve at 232 nm with a Fisher certified, nickel atomic absorption standard (1000 μg/ml, Fisher Scientific Co.).

Streptococcal Infectivity

For all infectivity exposures, mice were randomized and assigned equally to either a 0- or 24-hr test group. At the appropriate time, the 0- and 24-hr nickel-exposed and control groups were combined, placed in a Rochester-type stainless-steel chamber (Young and Bertke Co., Cincinnati, Ohio), and exposed to an aerosol of viable *Streptococcus pyogenes* (Group C). The bacterial aerosol was generated from a 24-hr Todd–Hewitt broth (THB, Baltimore Biological Laboratories, Cockeysville, Md.) culture of *S. pyogenes* (approximately 2×10^9 streptococci) for a 20-min period using a DeVilbiss atomizer (No. 40, DeVilbiss Co., Somerset, Pa.).

Animals were separated into their appropriate treatment groups and were maintained in clean, filtered, room air for 15 days. Total cumulative mortality rates were monitored and determined for $NiCl_2$- or $NiSO_4$-exposed and untreated control mice. The mortality data are presented as percentage mortality difference from control and relative mean survival time.

Streptococcal Clearance, Deposition, and Septicemia

Immediately following the streptococcal aerosol exposure, either two or four control animals were sacrificed for total lung streptococcal retention/deposition determinations. All aseptically excised lungs were homogenized in 1.8-ml sterile THB using a Sorvall homogenizer (Model Omni-Mixer, DuPont Instruments, Newton, Conn.) and individual stainless-steel microchamber homogenizer cups (10 ml, No. 18060). All homogenizations were performed in an ice bath for 3-min duration.

Quantitation of viable *in vivo* streptococci following infection was performed on excised lungs at various time intervals using the above procedure. Aliquots of lung homogenates incubated for 48 hr at 37°C on triplicate sheep blood supplemented (5%) Trypticase Soy agar plates (TSA, BBL) provided direct quantitation of viable streptococcal colony-forming units.

Septicemia determinations were performed on survivors of the mortality studies from blood cultures obtained by aseptically transverse sectioning mouse tails with scissors. The first drop of blood obtained in all cases was removed by wiping with surgical gauze saturated with 70% ethanol. Duplicate 0.05-ml samples were applied to TSA plates containing 5% sheep blood and streaked according to standard microbiological procedures. Following a 48-hr incubation period at 37°C, the percentage positive blood cultures were determined.

Pulmonary Cell Culture

Following 2-hr exposure to a $NiCl_2$ aerosol, rats were sacrificed with 150 mg Nembutal. Free cells of the lung from both exposed and untreated control animals were isolated and quantitated using previously described techniques (Coffin *et al.*, 1968); these techniques were also used with modifications for the smaller animal.

The *in situ* lung lavage was performed using prewarmed sterile 0.85% physiological saline at 37°C. The first 5.0-ml volume instilled into the rat lungs remained approximately 2 to 5 min; three subsequent 5.0-ml instillations were aspirated, withdrawn immediately, and pooled. The pooled cellular fraction was washed three times by centrifugation (365 *g*) and resuspended with a 1.0-ml volume of Medium 199 (Grand Island Biological Co., Grand Island, N.Y.; GIBCO) supplemented with 10% fetal bovine serum (GIBCO). The pulmonary cell culture was maintained in an ice bath during all subsequent manipulations. The cellular composition of the harvested cells from the pooled lavage fluid was determined from Wright-stained smears. Viability determinations were performed using the trypan blue dye exclusion method (0.4% trypan blue, GIBCO; Hanks and Wallace, 1958). Total cell count yields were determined by hemocytometer counting.

Measurement of Phagocytic Activity

Phagocytic activity of pulmonary cells obtained from rats following 2-hr exposures to a $NiCl_2$ aerosol was compared with untreated animal responses. Polystyrene latex spheres (1.1 μm, Dow Diagnostics, Indianapolis, Ind.) were used with the pulmonary cells cultured in Lab-Tek four-chamber microslides (Miles Laboratories, Inc., Naperville, Ill.) or in siliconized 25-ml Erlenmeyer flasks. Both systems maintained approximately 50 to 100 particles/cell in 1-ml volumes of the supplemented medium. All subsequent manipulations, including incubation conditions and determination of phagocytic activity, were performed according to procedures previously reported (Waters *et al.*, 1975b; and Gardner *et al.*, 1973).

Variations in phagocytic activity between attached versus suspension cell cultures were measured. Flask suspension cultures contained 3.0 ml of the pulmonary cell culture suspensions and the 1.1-μm latex spheres and were tested as previously reported (Gardner *et al.*, 1973). Each system, with appropriate controls, was tested in duplicate. All air-dryed Lab-Tek slides and smear slides from suspension cultures were treated with xylene (Fisher Scientific Co.) for 1 hr to remove extracellular latex spheres (Gardner *et al.*, 1973).

RESULTS

Pulmonary Deposition of Nickel in Mice

The pulmonary deposition of Ni following 2-hr inhalations of $NiCl_2$ atmospheres is shown in Fig. 1 for various aerosol concentrations ranging from 0 to 1000 μg Ni/m^3. The average values plotted in Fig. 1 are given with their standard errors. Regression analysis indicated a statistically significant increasing linear relationship ($P < 0.0001$, with an R^2 of 49%) between μg Ni/g dry lung and Ni aerosol concentration for mice sacrificed immediately after exposure.

Time Clearance of Inhaled Nickel from Mice Lungs

The ability of the murine lung to clear deposited nickel following 2-hr inhalations of a $NiCl_2$ atmosphere (583.73 $\pm$ 10.89 μg Ni/m^3) is given in Fig. 2. This $NiCl_2$ aerosol concentration was selected as appropriate for study based on results from the streptococcal infectivity experiments. Regression analysis indicated a statistically significant clearance ($P < 0.001$ with an R^2 of 77%) of the inhaled nickel over the 7-day postexposure assay period. The average values plotted in Fig. 2 are given with 95% confidence limits. Background nickel was determined in untreated control mice lungs to be 0.911 $\pm$ 0.13 μg Ni/g dry lung.

TABLE 1
INFLUENCE OF NICKEL INHALATION ON MURINE SUSCEPTIBILITY TO STREPTOCOCCAL INFECTION

Nickel aerosol concentration (μg Ni/m^3)[a]	Number of mice tested	Post treatment infection interval (hr)	Percentage mortality (difference from control)[a]	Relative mean survival time (days different from control)[a]
$NiCl_2$ exposures				
292.61 ± 6.49	120	0	6.25 ± 7.74	−0.57 ± 0.66
288.75 ± 6.39	100	24	3.74 ± 9.80	−0.46 ± 0.87
369.14 ± 26.47	80	0	6.25 ± 9.20	−0.64 ± 1.12
369.14 ± 26.47	80	24	6.25 ± 6.25	−0.76 ± 0.59
510.61 ± 21.56	160	0	13.67 ± 10.60	−0.44 ± 2.03
499.24 ± 31.81	120	24	26.04 ± 8.87[b]	−2.73 ± 0.78[b]
$NiSO_4$ exposures				
455.17 ± 24.90	120	0	4.25 ± 8.18	−0.25 ± 0.75
455.17 ± 24.90	120	24	20.83 ± 8.21[b]	−2.01 ± 0.79[b]

[a] Values are mean ± 1 SE.
[b] Mean values are significantly different ($P < 0.05$) from control.

pared to other treatment conditions, following inhalation of 499.24 ± 31.81 μg Ni/m^3 and infection after 24-hr recovery in clean air (Table 1). A similar response was observed following $NiSO_4$ exposure at 455.17 ± 24.90 μg Ni/m^3 and infection after 24-hr recovery in clean air (Table 1). These same conditions led to a significant decline in relative mean survival time. The number of mice tested in each group is also indicated in Table 1.

Control animals infected simultaneously with nickel-exposed mice demonstrated 21.23 ± 1.96% mortality and a 13.17 ± 0.17 day relative mean survival rate throughout these studies.

Clearance and Growth of Inhaled Streptococci

The toxic effects of nickel inhalation are further indicated in Table 2. Mice were exposed to a high $NiCl_2$ aerosol concentration (656.8 ± 50.3 μg Ni/m^3) comparable to that used in the previously described nickel clearance and streptococcal infectivity studies. Nickel-exposed mice demonstrated a reduced ability to clear inhaled streptococci as compared to infected control animals, especially at 72- and 96-hr postinfection. Control mice demonstrated a clearance trend typical of infected animals exhibiting the mortality pattern reported previously.

Growth of streptococci continued in the nickel-exposed mice and reached a level of 10^6 to 10^7 viable organisms per lung after 96 hr.

The severity of these effects would be expected to increase with higher levels of nickel via inhalation exposure as inferred from the data presented previously (Table 1).

The relationship between mortality and septicemia following streptococcal infection is shown in Fig. 3. As stated previously, mice infected 24 hr after exposure to a 656.8 ± 50.3 μg Ni/m^3 $NiCl_2$ aerosol demonstrated an enhanced mortality rate over infected control animals. Development of septicemia, measured by occur-

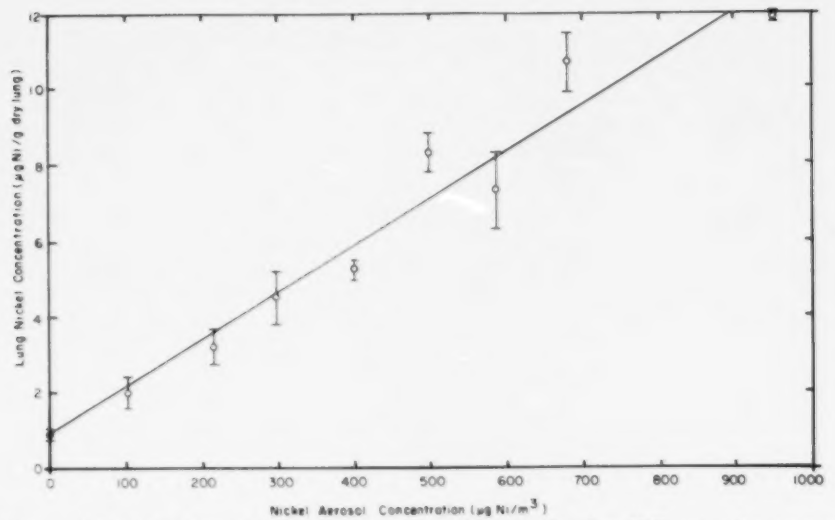

FIG. 1. Nickel deposition in lungs immediately after inhalation of various $NiCl_2$ aerosol concentrations by mice.

Streptococcal Infectivity Experiments

Following 2-hr exposures to various aerosols of nickel(II) salts, mice were infected either immediately or after 24-hr recovery in clean air. The mortality difference between $NiCl_2$-exposed and control mice increased significantly, com-

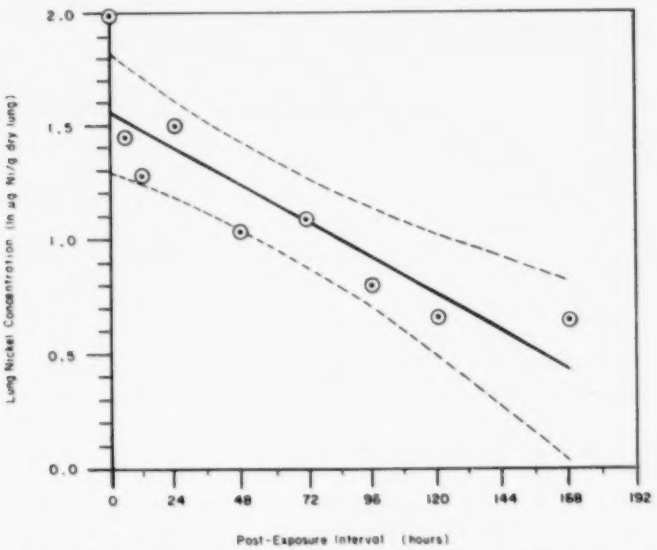

FIG. 2. Clearance of nickel from mice lungs following inhalation of a $NiCl_2$ atmosphere for 2 hr.

TABLE 2
CLEARANCE AND/OR GROWTH OF *Streptococcus pyogenes* IN MOUSE LUNGS FOLLOWING INHALATION ON NICKEL CHLORIDE

Post infection interval (hr)	Animal group: Nickel-treated (656.8 ± 50.3 μg Ni/m³) No. of mice	Nickel-treated Total number viable lung streptococci, $\log_{10}$[a]	Infected control No. of mice	Infected control Total number viable lung streptococci, $\log_{10}$[a]
0	19	3.02 ± 0.04	18	3.10 ± 0.03
2	10	2.53 ± 0.05	10	2.31 ± 0.04
4	19	2.33 ± 0.06	19	1.68 ± 0.14
6	10	1.43 ± 0.16	10	1.07 ± 0.17
24	9	3.43 ± 0.47	9	3.25 ± 0.51
48	7	4.89 ± 1.01	7	4.06 ± 1.02
72	24	5.67 ± 0.85	24	1.70 ± 0.48
96	15	6.22 ± 1.03	16	0.99 ± 0.27

[a] Values are means ± 1 SE.

rence of positive streptococcal blood cultures taken from survivors of the mortality studies, parallels the mortality rates. The occurrence of septicemia in the infected controls reflects those control animals that could not combat the infection, but were surviving at the time blood samples were taken.

Table 3 summarizes the cytotoxic effects of nickel inhalation on rat pulmonary cells. The data presented are expressed as percentage of control response following exposure to $NiCl_2$ aerosols. No differences between groups or systems regarding total cell yield and viability, both before and after the phagocytosis incubation period, were observed. Phagocytic capability was significantly depressed between groups ($P < 0.001$) in System 1 after 24 hr with the 1.1-μm spheres, but only slightly depressed between groups in System 2. The phagocytic response shown in System 2 was lower than that observed in System 1, perhaps because of lowered contact by alveolar macrophages with spheres in suspension.

The cellular composition of the pooled lavage fluid was determined in both control and nickel-exposed rats. Wright-stained smears indicated 98 to 99% alveolar macrophages, 1 to 2% lymphocytes, and no polymorphonuclear leukocytes in both groups, suggesting no differences in cell populations.

DISCUSSION

Inhalation exposures to nickel occur primarily in occupational areas such as mines, smelters, and various manufacturing operations (Wehner, 1975). Ambient-air nickel concentrations in urban areas (0.013 μg Ni/m³) are markedly higher than in nonurban areas (0.005 μg Ni/m³; Environmental Protection Agency, 1976). Occupational exposure to nickel is known to cause pulmonary health effects (Sullivan, 1969; Sunderman, 1970), however, the direct relationship between respiratory disease and exposure to nickel aerosols has not been reported.

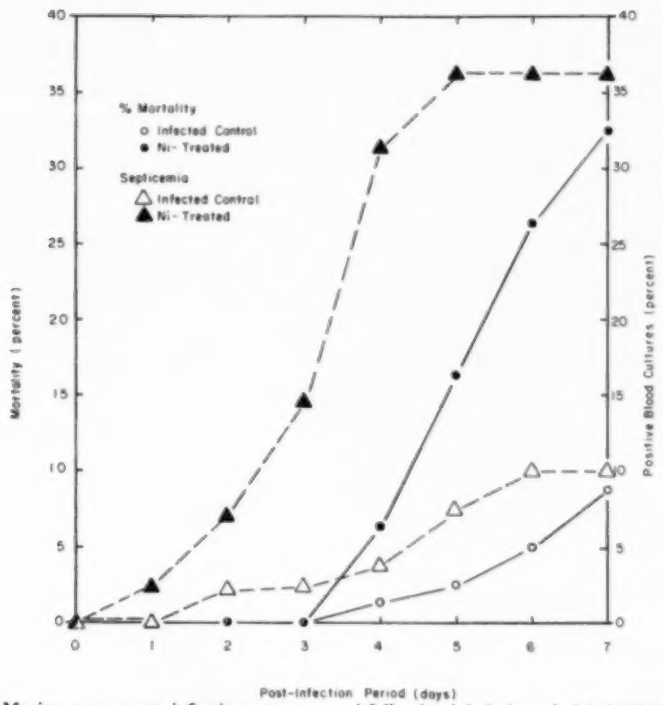

FIG. 3. Murine response to infectious streptococci following inhalation of nickel (656.8 ± 50.3 μg Ni/m³).

TABLE 3
EFFECTS OF NICKEL INHALATION ON RAT PULMONARY CELLS[a]

System[b]	Post exposure group	Cell number index[c]	Viability index[d]		Phagocytosis index[e] 1-μm spheres
			Before	After	
1	0 hr	88.3 ± 7.1	98.1 ± 3.8	100.6 ± 2.8	117.3 ± 12.4[f]
	24 hr	87.4 ± 14.0	97.4 ± 3.5	102.9 ± 1.5	80.9 ± 12.0
2	0 hr	86.2 ± 9.1	98.4 ± 2.6	94.8 ± 8.8	92.2 ± 18.8
	24 hr	87.4 ± 13.9	97.6 ± 3.2	100.9 ± 0.6	73.1 ± 5.6

[a] Values are means ± 1 SE.
[b] System 1 was attached cell culture system. System 2 was suspension culture system.
[c] Cell number index = total number of cells from nickel-treated animals/total number of cells from control animals (× 100).
[d] Viability index = (before and after the phagocytosis incubation period) percentage viability of cells from nickel-treated animals/percentage viability of cells from control animals (× 100).
[e] Phagocytosis index = percentage cells with spheres from nickel-treated animals/percentage cells with spheres from control animals (× 100).
[f] Means are statistically different ($P < 0.05$) than controls.

Several investigators have reported that nickel, as well as other metals, will alter several of the host's respiratory system defense mechanisms against infectious agents. At the ciliated epithelium level, nickel was shown to significantly depress normal ciliary activity in a hamster tracheal ring assay following *in vivo* exposure to 100 μg Ni/m^3 for 2 hr (Adalis *et al.*, 1978). In the alveolar region, continuous exposure to nickel oxide and nickel chloride was shown to enhance the number of free cells obtained from rats by saline endotracheal lavage following inhalation exposure (Bingham *et al.*, 1972). Acute exposure to nickel (20 hr) was demonstrated to be cytotoxic in an *in vitro* rabbit alveolar macrophage assay depressing cellular viability, phagocytic activity, and acid phosphatase activity (Graham *et al.*, 1975a; Waters *et al.*, 1975a). Nickel may also depress the immunocompetence of the host. Graham and co-workers (1975b) reported that nickel adversely affects splenic primary antibody production in mice at concentrations as low as 6.17 μg Ni/g. Nickel was found to be a potent inhibitor of antibody-mediated rosette formation by altering the F_c receptor site on pulmonary lung cells (Hadley *et al.*, 1977).

The inhalation toxicity of metals and increased susceptibility to infectious diseases are apparently attributable to a multitude of subtle effects. These factors were reported previously for cadmium (Gardner *et al.*, 1977) and are suggested in this study for nickel. In the infectivity system described here, nickel caused an enhancement in streptococcal infection at exposure concentrations $\geq$ 500 μg Ni/m^3, as evidenced by the similarity in response to nickel(II) salts. Increased susceptibility to induced pulmonary infections, mimicking increases in human respiratory diseases resulting from occupational exposure to various toxicants, is inferred here and warrants further epidemiological study.

Although the aerosol concentrations used in these studies were several times higher than those found in the working environment, it is important to recognize that these exposures were for 2-hr durations. Repeated daily exposure to lower concentrations may produce the same effect on host defense mechanisms and thus render the population at risk more susceptible to pulmonary disease.

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